FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

DEPARTMENT OF PHARMACOLOGY, TOXICOLOGY AND BIOCHEMISTRY
RESEARCH GROUP PHARMACOLOGY

FLUOROQUINOLONE RESISTANCE IN CLINICAL AVIAN PATHOGENIC ESCHERICHIA COLI ISOLATES FROM FLANDERS (BELGIUM)

Temmerman R., Garmyn A., Antonissen G., Vanantwerpen G., Vanrobaeys M., Haesebrouck F., Devreese M.

GHENT UNIVERSITY

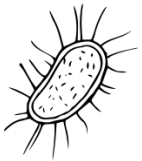
ECVPT
European College of Veterinary Pharmacology & Toxicology

DGZ
SAMEN VOOR GEZONDE DIEREN

CHAIR
POULTRY
HEALTH SCIENCES

Introduction – Objectives

Colibacillosis is one of the leading causes of **disease-related economic loss** in the **poultry sector**



Colibacillosis is caused by an infection of **avian pathogenic *Escherichia coli* (APEC)**

Fluoroquinolones, such as enrofloxacin (ENRO) are frequently used antimicrobials (via the drinking water) for the treatment of APEC infections in the poultry industry

However, development and selection of **resistance** to these antimicrobial drugs is an increasing problem

The goal of this study was to assess the **prevalence and mechanisms of resistance** against ENRO in clinical APEC isolates from Flanders.

- Determination of minimum inhibitory concentration (**MIC**) distribution of 125 clinical APEC isolates (gradient strip test)
- Determination of mutant prevention concentration (**MPC**) values of a sample of sensitive strains, i.e. MIC values of 0.016 and 0.032 µg/mL (agar dilution technique)
- Non-wild type (NWT) strains were further screened for the presence of **QRDR** (quinolone resistance determining regions) mutations (*gyrA, ParC, ParE*) and **PMQR** (plasmid mediated quinolone resistance) genes (*qnrA, qnrB, qnrS, oqxAB, qepA*) using PCR, gel electrophoresis and gene sequencing (Sanger)

Results: MIC and MPC

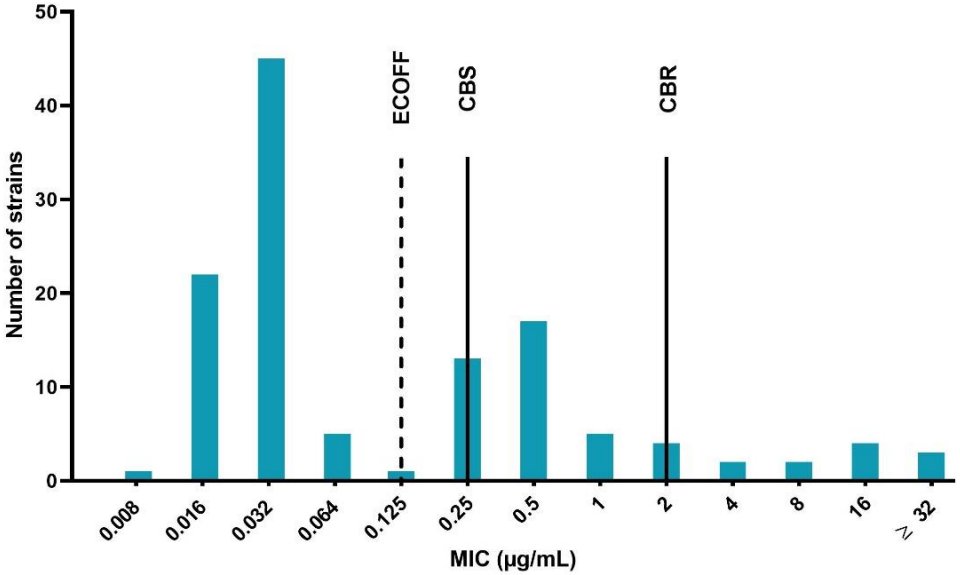
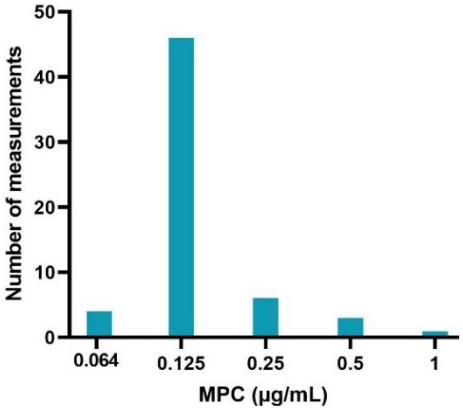


Figure (left). *Enrofloxacin MIC distribution of the 125 avian pathogenic Escherichia coli (APEC) strains isolated from broiler farms in Flanders with colibacillosis outbreaks. The dotted line represents the epidemiological cut-off (ECOFF), which is 0.125 µg/mL. Strains with MIC values ≤ ECOFF are labelled wild type (WT, 60% of the strains) and strains with higher MIC values than the ECOFF as non-wild type (NWT, 40% of the strains). The full lines marked CBS and CBD indicate the clinical breakpoint for susceptibility (0.25 µg/mL, CBS) and resistance (2 µg/mL, CBR), respectively. Strains with MIC in between CBS and CBR are designated intermediate (21% of the strains). Sixty-nine percent of the strains were susceptible and 10% resistant.*

Figure (right). *Enrofloxacin MPC distribution of 20 sensitive avian pathogenic* Escherichia coli *(APEC) strains obtained via agar dilution experiments, performed in triplicate (60 measurements in total).*



Results: QRDR and PMQR

MIC (µg/mL)	Number of isolates	QRDR mutations			PMQR
		gyrA	*parC*	*parE*	
0.25	1				*qnrS*
	12	S83L			
0.5	17	S83L			
1	2				*qnrS*
	2	S83L			
	1	S83L	S80R		
2	1				*qnrS*
	2	S83L			*qnrB*
	1	S83L	S80R		
4	2	S83L			*qnrS*
8	1	S83L			*qnrS*
	1	S83L/D87N	S80R		
16	4	S83L/D87N	S80I		
≥ 32	1	S83L/D87N	S80R	S458A	
	2	S83L/D87N	S80I	S458A	

Table. *Overview of the fluoroquinolone resistance mechanisms (QRDR and PMQR) of the different NWT strains (strains with MIC values above the ECOFF). Of all the NWT strains (total 50) :*

- *92% carried one or more mutations in gyrA.*
- *ParC mutations occurred in 22% of the cases*
- *ParE mutations were rare (6% or 3 strains)*
- *18% possessed PMQR associated genes. Most detected was qnrS (14%), followed by qnrB (4%). The other investigated genes were not detected.*

Conclusion

- **MIC results:** A large number of strains (40%) were considered NWT. In contrast to the relatively high number of NWT strains, only a minority of this group was designated as resistant (R), indicating the presence of a considerable intermediate portion of strains (21%).
- **MPC results:** MPC values of the tested isolates were fairly stable across the different. In 76.67% of the cases, the MPC was 0.125 µg/mL. Approximately 17% of the measurements had higher MPC values. Two strains exhibited a MPC/MIC ratio of 32 (MPC 0.5 and 1 µg/mL, respectively), indicating a large mutant selection window (MSW). A wide MSW increases the risk of selective enrichment of resistant subpopulations in a bacterial culture.
- **QRDR/PMQR results:** Chromosomal mutations in DNA gyrase and topoisomerase IV are the main source of fluoroquinolone resistance in *E. coli*, deemphasising the role of PMQR mechanisms.

FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

Robin Temmerman

DVM, PhD Student, Resident ECVPT

LABORATORY OF PHARMACOLOGY AND TOXICOLOGY

E robin.temmerman@ugent.be

T +32 4 98 18 01 78

Ghent University

@ugent

Ghent University

www.ugent.be

GHENT UNIVERSITY